General stress proteins: Novel function and signals for induction of stationary phase genes in *E. coli*

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AKADEMISK AVHANDLING

För filosofie doktorsexamenen i mikrobiologi (examinator Thomas Nyström), som enligt fakultetssyrelsens beslut kommer att offentligt försvaras tisdagen den 25 maj 2010, kl. 10.00 i föreläsningssal Arvid Carlsson, Medicinaregatan 3, Göteborg

Fakultetsopponent : Professor Susan T. Lovett, Brandeis University, Massachusetts, USA

Papers included in this thesis;

I. Metabolic control of the *Escherichia coli* universal stress protein response through fructose-6-phosphate. **Persson Ö**, Valadi A, Nyström T, Farewell A. Mol Microbiol. 2007 65(4):968-78.

II. The levels of fructose-6-phosphate regulate σ^S-dependent transcription upon entry to stationary phase in *E. coli*. **Persson, Ö**, Gummesson, B., Hallberg, E., Lilja, E., and Farewell, A. Manuscript

III. Decline in ribosomal fidelity contributes to the accumulation and stabilization of the master stress response regulator σ^S upon carbon starvation. Fredriksson Å, Ballesteros M, Peterson CN, **Persson Ö**, Silhavy TJ, Nyström T. Genes Dev. 2007 21(7):862-74.

IV. UspB, a member of the sigma-S regulon, is required for RuvC-dependent resolution of Holliday junctions. **Persson, Ö**, Nyström, T and Farewell, A. Under revision DNA Repair.

ABSTRACT

Survival during conditions when nutrients become scarce requires adaptation and expression of genes for maintenance in order for the cell to survive. Among the numerous proteins involved in adaptation and regulation under these conditions, the stationary phase sigma factor, σ^S, and the Universal stress proteins contribute to survival and bestow the cell with general stress protective functions during growth arrest. In this work we found new mechanisms for the cell to prepare and sense the intracellular environment and respond accordingly.

The *usp* genes and the *rpoS* gene (encoding σ^S) were found to be positively regulated by metabolic intermediates of the glycolysis in the central metabolic pathway. Specifically, mutations and conditions resulting in fructose-6-phosphate accumulation elicit superinduction of these genes upon carbon starvation, whereas genetic manipulations reducing the pool size of fructose-6-phosphate have the opposite effect. Under carbon starvation, transcription of the *usp* and the *rpoS* genes require and are modulated by the alarmone ppGpp. The observed positive transcriptional regulation by fructose-6-phosphate is not via alterations of the levels of ppGpp. None of the known regulators examined were found to be required for the superinduction. We suggest a novel regulatory mechanism involving the phosphorylated intermediates as a signal molecule for monitoring and subsequent regulation of stress defense genes. Based on mutational studies we also suggest that this signaling mechanism secures accumulation of required survival proteins preceding the complete depletion of the external carbon source.

Entry into stationary phase promotes a dramatic stabilization on the sigma factor σ^S. Mistranslated and oxidized proteins were shown to contribute to elevated levels of σ^S and transcription of its regulon. Furthermore, ribosomal alleles with enhanced translational accuracy attenuate induction of the RpoS regulon and prevent stabilization of σ^S. Destabilization of σ^S is governed by the ClpXP protease, for which aberrant proteins also are substrates. Mechanistically, generation of mistranslated proteins by starvation, or other means, competes for the common enzyme for degradation, and thereby sequesters the pool in favor of σ^S stabilization.

A growing body of evidence shows that there is an intimate connection between proteins required for genome stability and stationary phase survival. We show that the integral membrane protein UspB, a member of the RpoS regulon, is required for proper DNA repair as mutants lacking *uspB* are sensitive to several DNA damaging conditions. Genetic and biochemical studies demonstrate that UspB acts in the RuvABC recombination repair pathway and removing *uspB* creates a phenocopy of the DNA resolvase mutant, *ruvC,* which includes a reduced efficiency in resolving Holliday junctions. Further, we show that the *uspB* mutant phenotype can be suppressed by ectopic overproduction of RuvC and that both *ruvC* and *uspB* mutants can be suppressed by inactivating *recD*. The fact that RuvABC-dependent repair requires UspB for proper activity suggests that the σ^S-regulon works together with DNA repair pathways under stress conditions to defend the cell against genotoxic stress.

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